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1053-Pos Board B808

Protein Immobilization on Chemically Functionalized Germanium Investigated by ATR-FTIR

Jonas Schartner, Jörn Güldenhaupt, Klaus Gerwert, Carsten Kötting.
Biology & Biotechnology, Bochum, Germany.

The attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR) allows a detailed analysis of surface attached molecules, including their secondary structure, reaction mechanism, orientation and interaction with small molecules or proteins [1]. The aim of our study is the development of a universal immobilization technique on germanium for proteins with different tags or residues (e. g.: his-tag, strep-tag, cysteine, amine, etc.). The first step was the organic synthesis of triethoxysilanes with modified functional head groups (e. g.: maleimido, keton, succinimidyl-ester). The attachment of the triethoxysilanes on germanium was monitored by ATR-FTIR and X-ray-photoelectron-spectroscopy [2]. By a reaction of the succinimidyl-ester with amino-nitrilotriacetic acid a carboxylic terminated surface, which coordinates divalent cations like Ni2+, was created. As an example, the small GTPase Ras was attached to the carboxylated surface via his-tag [3]. The cellular function of active Ras as molecular switch was shown by a berylliumfluoride titration assay, which allows the on and off switching of Ras at atomic resolution by means of difference-spectroscopy [3]. The treatment with imidazole removes over 80 % of the attached proteins and allows a repeated binding of Ras [3]. Furthermore, the activity of immobilized Photosystem I (PSI) was proven by light-induced difference spectroscopy [3]. In comparison with recent techniques, the attachment and analysis of transmembrane proteins becomes accessible, because measurements in detergent are now possible. Further advantages are the reusability of the surface, a better signal to noise ratio and the covalent functionalization of germanium, which leads to a more stable attachment.

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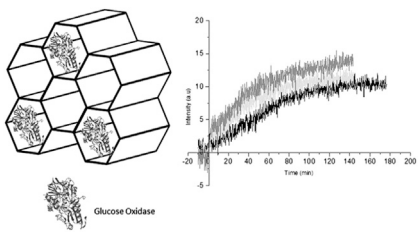
1054-Pos Board B809

Monitoring the Kinetics of Enzyme Immobilization into Mesoporous Silica by Real Time Fluorescence

Pegah Sadat Nabavi Zadeh, Nils Carlsson, Kassam Abdel Mallak, Björn Åkerman.
Chalmers univeristy of technology, Göteborg, Sweden.

Mesoporous silica particles are used for immobilization of enzymes in order to increase enzyme stability, facilitate product purification and reuse of enzyme. An important question is how the size of the enzyme affects the rate of immobilization and also whether the immobilization rate depends on silica particle concentration.

Here, we examine the immobilization of three enzymes with different size. The enzymes used are Lipase, Bovine serum albumin (BSA), Glucose oxidase (Gox) and the diameter of them, respectively, are 4nm, 7nm, and 9nm; while the diameter of the silica particle pores is 9nm. In this research fluorescence spectroscopy is used as a direct monitoring technique. The immobilization is followed by recording the fluorescence from epicocconone, a dye binding to the enzyme. The results show that there is a relation between the size of enzyme and the immobilization rate, the larger the enzyme the slower the rate of immobilization. Also the investigation shows that by increasing mesoporous silica concentration the immobilization rate increases. Further, real-time data on immobilization rate and the enzyme and particle concentration dependence can be used to test models for the immobilization process.

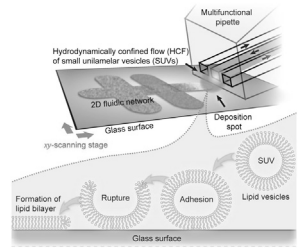


1055-Pos Board B810

Lab on a Biomembrane

Alar Ainla, Irep Gözen, Bodil Hakonen, Aldo Jesorka.
Department of Chemical and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden.

Lipid bilayer membranes are among the most ubiquitous structures in the living world, with intricate structural features and a multitude of biological functions. It is attractive to recreate these structures in the laboratory, as this allows mimicking and studying the properties of biomembranes and their constituents, and to specifically exploit the intrinsic two-dimensional fluidity. Even though diverse strategies for membrane fabrication have been reported, the development of related applications and technologies has been hindered by the unavailability of both versatile and simple methods. Here we report a rapid prototyping technology for two-dimensional fluidic devices, based on in-situ generated circuits of phospholipid films. In this ''lab on a molecularly thin membrane'', various chemical and physical operations, such as writing, erasing, functionalization, and molecular transport, can be applied to user-defined regions of a membrane circuit. This concept is an enabling technology for research on molecular membranes and their technological use.



1056-Pos Board B811

Lipid Nanodomains on Modified Gold Surfaces - A Biomimetic Platform to Study Electroactive Biomolecule-Membrane Interactions

Joaquim M. Trigo Marquês, Ana S. Viana, Rodrigo F.M. de Almeida.
Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal.

Biomimetic platforms based on supported lipid bilayers (SLB) on gold are becoming prominent due to their wide applications in biosensor developing and redox biology studies. A recent innovation was the use of multicomponent lipid bilayers with phase coexistence (Marquês et al. 2013). Here, the formation of ternary (DOPC/DPPC/Cholesterol 2:2:1) SLBs exhibiting lipid rafts in modified gold will be described. Surface modifications are required to confer hydrophilicity to gold facilitating the formation of a planar and uniform bilayer. Two surface modifications, 11-mercaptoundecanoic acid (MUA) which generates a long and compact self-assembled monolayer (SAM) and L-cysteine (Cys) which forms a short and non-compact SAM, were used. Similar kinetics was observed for lipid vesicle adsorption on both SAM by surface plasmon resonance and quartz crystal microbalance. On both modified electrodes a continuous lipid bilayer with topographical details assigned to lipid rafts was imaged by atomic force microscopy. Moreover the thickness estimated by ellipsometry lies in the range expected for a bilayer.

Since the compact MUA monolayers hinder the electronic exchange between electroactive molecules and the gold electrode, Cysteine emerges as a suitable solution to develop lipid-based interfaces to study electrochemical phenomena. Cys-modified electrodes with raft-containing SLB were employed to study membrane-interactions of a redox active biomolecule, epinephrine. Cyclic voltammetric results revealed an influence of lipid composition on the membrane-adsorbed epinephrine. Interestingly, fluorescence spectroscopy revealed a rather weak interaction between epinephrine and lipid bilayers in suspension, highlighting the applicability of the platform cys-SLB to investigate and detect membrane-associated processes with high sensitivity.

Marquês, JT, de Almeida, RFM, Viana, AS, 2013, *Electrochim. Acta* (in press).

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1057-Pos Board B812

Direct Measurement of Protein Translocation across Droplet Interface Bilayers

Matthew A. Holden.
University of Massachusetts, Amherst, MA, USA.

We introduce a new method for monitoring and quantitating the transport of materials across a model cell membrane. As a proof of concept, we investigate how a small cell-penetrating peptide called Pep-1 is able to carry a large object,

such as a protein, across a lipid bilayer. To create a membrane, two sub-microliter, lipid-encased aqueous droplets are contacted - termed a droplet interface bilayer (DIB). The peptides adsorb to the protein cargo non-covalently and somehow ''carry'' the protein from one droplet to the other through the membrane. We then assay the translocated cargo through a fluorogenic assay. The DIB method recapitulates the findings of earlier studies involving Pep-1, including the dependence of protein transport on voltage and membrane charge, while also contributing new insights. Specifically, we found that the symmetry of the bilayer membrane may play a role in Pep-1-mediated protein translocation. In addition, we used a newly developed peptide transduction domain mimic (PTDM) as a protein carrier, which exhibited distinct differences compared to Pep-1's mechanism. We've also used the DIB system to monitor the translocation of proteins through pores, such as the anthrax toxin. We anticipate that the DIB method may be useful for a variety of transport-based studies; in particular those which must make use of tiny quantities of purified species.

1058-Pos Board B813

Cobaltabisdicarbollide Macroanion is able to Diffuse across the Lipid Membrane; Study of Kinetics and Transport

Carmina Verdiá Báguena[1], Antonio Alcaraz[1], Clara Viñas[2], Francesc Teixidor[2], Vicente M. Aguilella[1].
[1]Universitat Jaume I, Castellón de la Plana, Spain, [2]2Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Barcelona, Spain.

The ability to form membranes is not restricted to polar lipid molecules or even to organic molecules, as the boron-based molecule cobaltabisdicarbollide, $[Co(C_2B_9H_{11})_2]^-$, (commonly known as cosan) can also form vesicles and membranes. Here we show that cosan and its derivatives can interact with phospholipid membranes.

Cosan is a monoanionic metallabisdicarbollide, which is soluble in both water and oils. Structurally, cosan has no similarity to the polarized lipid molecules that make up biological membranes. It comprises a cobalt atom sandwiched by two carboranyl clusters [1-3]. Although these clusters are hydrophobic, the metal ion imparts a dispersed negative charge spread over the whole molecule (fig.1). As a consequence of this, the exposed B-H and C-H bonds of the carboranyl clusters possess weak dipoles and form intermolecular attractions that give the molecule its unusual physico-chemical property of being simultaneously hydrophobic and hydrophilic. This duality allows cosan to interact with lipid membranes.

Using membrane electrophysiology recordings and direct measurements of COSAN concentrations by inductively coupled plasma mass spectrometry (ICP-MS) we show that COSAN transits cell-free artificial lipid membranes. We study the kinetics of the transport and try to get insights on the mechanism by which COSAN crosses planar phospholipid membranes.

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1059-Pos Board B814

Experimental Observation of Surface Charge Inversion in a Biological Nanopore in Presence of Monovalent and Multivalent Cations

María L. López-Peris, María Queralt-Martín, Vicente M. Aguilella, **Antonio Alcaraz**.
Physics, University Jaume I, Castellon, Spain.

The electric double layer formed at the pore surface produces the exclusion of coions and the accumulation of counterions. A particularly captivating situation occurs when interfacial charges attract counterions in excess of their own nominal charge, thus leading to an effective charge inversion of the system. This phenomenon has been reported in such diverse systems like lipid vesicles, colloids, Langmuir monolayers, membranes, flexible polyelectrolytes and other synthetic nanodevices that are in contact with an aqueous solution containing multivalent ions. Here, we report experimental evidence of charge inversion in the bacterial channel OmpF of E. coli, not only in presence of multivalent cations but also in presence of monovalent ones. We perform reversal potential experiments in different conditions of pH and salt concentration to analyze both the effect of cation type and size in the selectivity changes.

1060-Pos Board B815

Influences on Cellular Adhesion of Nanoparticles under Blood Flow-Like Conditions

Ellen Broda, Ulrich Lächelt, Frauke Mickler, Ernst Wagner, Christoph Bräuchle.
LMU München, München, Germany.

Nanocarrier-mediated drug and gene delivery are novel strategies to treat diseases, for example cancer, neurological disorders, infectious and cardiovascular diseases. Sophisticated drug delivery and gene therapy systems are typically equipped with targeting ligands to improve the specific binding to diseased cells within the human body. These targeting moieties are usually small molecules, peptides or proteins which are able to specifically bind receptors on the cell surface. In many diseased cells certain receptors are overexpressed and therefore represent suitable targets.

In our experiments we focus on the specific adhesion of nanocarriers to target cells. Therefore, we use fluorescent labeled polystyrene beads as model particles, shield them with PEG (polyethylene glycol) and mount a ligand - the transferrin receptor (TfR) binding peptide B6 - to mimic the surface of therapeutical nanocarriers. The binding of these targeted beads is directly compared to the adhesion of non-targeted beads on TfR overexpressing HuH7 cells. As non-targeted beads we use three different types, one with hydroxyl groups on the surface, another with scrambled B6 peptide (same amino acids, but different order) and a third type with modified B6 peptide (all positively charged amino acids are replaced by neutral ones). To include dynamics and determine the impact of shear stress, the binding study is performed under laminar flow conditions, i.e. the beads are flushed over a cell monolayer within a microfluidic channel. After fixation of the cells, highly-sensitive fluorescence widefield microscopy is performed to analyze the adhesion of beads on a single cell level. With this approach we are able to directly analyze the effect of the targeting ligand. In addition, the influences of electrostatics and shear stress on cellular particle binding are investigated.

1061-Pos Board B816

Surface Interactions in Suspensions of Swimming Cells

Vasily Kantsler[1], Jorn Dunkel[2], Raymond E. Goldstein[3].
[1]Department of Physics, University of Warwick, Coventry, United Kingdom, [2]Department of Mathematics, Massachusetts Institute of Technology, Cambridge, MA, USA, [3]DAMTP, University of Cambridge, Cambridge, United Kingdom.

Interactions between swimming cells and surfaces are essential to many microbiological processes, from bacterial biofilm formation to human fertilization. However, despite their fundamental importance, relatively little is known about the physical mechanisms that govern the scattering of flagellated or ciliated cells from solid surfaces. A more detailed understanding of these interactions promises not only new biological insights into structure and dynamics of flagella and cilia but may also lead to new microfluidic techniques for controlling cell motility and microbial locomotion, with potential applications ranging from diagnostic tools to therapeutic protein synthesis and photosynthetic biofuel production. Due to fundamental differences in physiology and swimming strategies, it is an open question of whether microfluidic transport and rectification schemes that have recently been demonstrated for pusher-type microswimmers such as bacteria and sperm cells, can be transferred to puller-type algae and other motile eukaryotes, because it is not known whether long-range hydrodynamic or short-range mechanical forces dominate the surface interactions of these microorganisms. Here, using high-speed microscopic imaging, we present direct experimental evidence that the surface scattering of both mammalian sperm cells and unicellular green algae is primarily governed by direct ciliary contact interactions. Building on this insight, we predict and experimentally verify the existence of optimal microfluidic ratchets that maximize rectification of initially uniform Chlamydomonas reinhardtii suspensions. Because mechano-elastic properties of cilia are conserved across eukaryotic species, we expect that our results apply to a wide range of swimming microorganisms.

1062-Pos Board B817

Selective Growth of Neural Networks on Micro-Patterned Graphene

Sandeep Keshavan[1], Matteo Lorenzoni[1], Fernando Brandi[1], Andrea Giugni[2], Francesca Cella Zanacchi[1], Bruno Torre[1], **Silvia Dante**[1].
[1]Nanophysics, Istituto Italiano di Tecnologia, Genova, Italy, [2]Nanostructures, Istituto Italiano di Tecnologia, Genova, Italy.

Single crystal graphene is the ideal candidate for next generation of electronic devices and of biosensors. Interfacing graphene with neural cells could be highly advantageous in exploring their electrical behaviour and promising for several biomedical applications, including neural regeneration and artificial retina. Here, we present a straightforward fabrication technique based on laser ablation to obtain patterned substrates promoting ordered neuron growth. Chemical vapor deposition (CVD) single layer graphene (SLG) was machined by means of single pulse UV laser ablation technique at the lowest effective laser fluence in order to minimize laser damage effects (1). The obtained patterned substrates, with alternating micro-sized stripes of ablated and not-ablated SLG, were uniformly coated with poly-D-lysine; primary embryonic hippocampal neurons were cultured on the functionalized substrates. As monitored by time-lapse imaging, neurons adhered on both regions of the pattern,